LIQUID-LIQUID EXTRACTION PROCESS FOR HIGH EFFICIENCY RAPID PURIFICATION OF COPPER RADIONUCLIDES

by

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According to a new market report published by Transparency Market Research, the global market for radiopharmaceuticals was valued at approximately 3.8 billion USD in 2011 and is expected to reach 12.2 billion USD by 2018 (2013). Usually, these radiopharmaceuticals consist of three major components: 1) a biomolecule (BM) responsible for receptor targeting; 2) a bifunctional chelate (BFC) for radionuclide coordination; and 3) a radionuclide for imaging and/or radiotherapy. Specific activity (SA) is one of the most important parameters for characterizing the quality of radionuclides or radiotracers. In many cases, it is the limiting factor for using radiotracers in biological systems, especially when targeting low capacity receptors, or when there is a risk of inducing pharmacological side effects. Competition for the low abundance receptors can arise not only from unlabeled versions of the tracer molecule, but also from other molecules of related structures, such as byproducts from radiolabeling procedures. Therefore, achieving high SA is extremely important for radiopharmaceuticals that target proteins, such as tumor receptors, that are present in very low (nM or less) concentrations *in vivo* (Woods, Wiseman et al. 1980, Velikyan, Beyer et al. 2004, Velikyan, Beyer et al. 2008, Patil, Gada et al. 2012, Zeng, Lee et al.

2012). However, one of the major barriers to improving the effective Specific Activity of radiometals is contamination from a variety of non-radioactive metal contaminants. In addition to optimizing radiolabeling reactions and/or separation methods, removing the metal contaminants from radiometals is a direct and efficient approach to increasing the effective SA of metal-based radiotracers. A water oil separation device was created for the purpose of purifying Cu-64, for the application of the preparation of promising radiopharmaceutical compounds for diagnosis and radiotherapy. The membrane utilized in the device is capable of separating the aqueous solution from the organic solvent. Based on the data obtained from UV-Vis spectroscopy of the separated two phases that contain different colored dyes, the purity of either the water or the organic phase separated by the membrane is greater than 99 %.

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PREFACE

This thesis is ultimately based on a proof of concept for a technology/technique that will require quantitative analysis to account for the efficiency of the process. Qualitative research was carried out to ensure that the concept behind this research was feasible and plausible for future students to carry it out.

I would like to thank my mentors Dr. Gao and Dr. Zeng for all they taught me and especially for their patience with me during my transition from a PhD student to a Master student. I especially want to thank the post doctorate student in Dr. Gao's lab, Dr. Jiamin, for all the relentless help he gave me. He was a kind hearted individual and I wish him God's blessings on his future endeavors. I would like to thank my family: Mommy and Daddy Okoye, Afoma Okoye and Ujunwa Okafor, friends: Bolanle Odusanya, Elizabeth Adewale, Natasha Mishkova and my Love, Kolawole Daramola; for their endless support while I was in school. I am surrounded by great friends and family members and I thank God for that. I appreciate their prayers, encouragement and love. "A friend loves at all times, and a brother is born for a time of adversity" (Proverbs 17:17). I especially want to thank God for Jesus Christ and the Holy Spirit because without God working in me and around me, I would be nothing. John 15:5 Jesus is the vine and I am the branch, apart from him I can do nothing.

1.0 INTRODUCTION

Positron emission tomography (PET) is a clinically applicable non-invasive technique for imaging the expression of successful gene transduction in specific organs of the human body. PET imaging could be used to define the magnitude, location and persistency of gene therapy in the human body (Blasberg and Tjuvajev 2003). PET can aid in studying the effects of cancer therapy in the human body, and can be used as a tool to indicate whether or not a certain treatment is successful. Therefore, PET plays a vital role in properly diagnosing cancer or verifying suspected recurrences. Antibodies labeled with position emitting isotopes are preferable for PET due to the improved PET sensitivity (Anderson, Connett et al. 1992). Currently F-fluorodeoxyglucose FDG is one of the major radiotracers being used in the application of PET. The preparation of these PET radiopharmaceuticals is highly demanding. It requires precise manipulations due to the short half-life (^{18}F, $t_{1/2}$= 110 min), as well as an expensive automated apparatus and highly skilled personnel (Blasberg and Tjuvajev 2003). Another limitation of this current radiotracer is the molecule size. FDG is a small molecule, therefore it is easily taken up by benign lesions (inflammatory processes), and because FDG isn't as site specific, there are physiological variations with the distribution of FDG in the human body (Kircher, Hricak et al. 2012). PET- FDG has been studied to quantify the sensitivity, specificity and accuracy. Results showed that PET-FDG has a sensitivity of 89-100%, specificity of 69-100% and accuracy of 89-100% (Kircher, Hricak et al.

2012). Due to the issue of specificity of the FDG radiotracer, there is a need for a site-specific radiotracer.

Introduction of highly specific activity reactors and accelerator-produced isotopes has led to radionuclides being in demand. The office of Isotopes for Medicine and Research (OIMS) instituted a network of large reactors around the world (The virtual Isotope Center, VIC) for the production of radionuclides in high demands on a yearly basis (Srivastava and Dadachova 2001). These factors contribute to the radionuclide being readily available for use in research for further understanding.

In the last decade research has been carried out to study the advancing field of radionuclide therapy. This field is quickly growing due to improved bioengineering vehicles for delivering radionuclides to tumors (Srivastava and Dadachova 2001). Some radionuclides that can be applied in PET are zirconium-89 ($t_{1/2}$= 78.4 h), bromine-76 ($t_{1/2}$ = 16.1 h), iodine-124 ($t_{1/2}$ = 4.15 d), copper-64 ($t_{1/2}$ = 12.7 h) (Anderson, Connett et al. 1992). ^{64}Cu is the radionuclide of interest for the purpose of this research, due to its intermediate half-life and its emission characteristics (Toyota, Hanafusa et al. 2012). ^{64}Cu has a β- of 37.1% and β+of 17.9%, due to its excellent solubility in water, ^{64}Cu is a potential for a radiotracer. High yield of ^{64}Cu increased and high specific activities can be achieved, using biomedical cyclotrons to generate the radionuclide (Matarrese, Bedeschi et al. 2010). ^{64}Cu can also be produced using a reactor, therefore, it can be readily available for use (Anderson, Connett et al. 1992). Anderson et al concluded that there is a viability of ^{64}Cu in the use of diagnosing colon cancer. This shows promise for the radionuclide if further studies can be completed with the radionuclide. When it comes to the application of ^{64}Cu, there is a need for the purification of the products of the cyclotron-produced radionuclide. This is because Fe^{3+}, Zn^{2+}, Ni^{2+}, Co^{2+} and non-radioactive Cu^{2+} are some of the major transition metal impurities that come

from the cyclotron-produced ^{64}Cu. If ^{64}Cu is not properly purified, the impurities may be harmful to human health. It will affect the labeling process for the application of ^{64}Cu as a radiotracer (Zeng, Lee et al. 2012).

With the increased availability of medical cyclotrons and improvements in cyclotron production, the use of "non-standard" radionuclides has also increased accordingly (Anderson and Welch 1999, Lewis, Welch et al. 2008, Zeng and Anderson 2013). Among those "non-standard" radionuclides, copper radionuclides, including ^{60}Cu, ^{61}Cu, ^{62}Cu, ^{64}Cu and ^{67}Cu, attract increased interest due to the broad applications in diagnostic imaging, radiotherapy and theranostics (Novak-Hofer and Schubiger 2002, Wadas, Wong et al. 2007, Anderson and Ferdani 2009, Smith, Bowers et al. 2012). The use of copper radionuclides presents a number of advantages, including: (i) copper is limited to two principle oxidation states (I and II) and the coordination and redox chemistry are well documented, (ii) the biochemistry and metabolism of copper is also well known; and (iii) long-term targeting and trapping is made feasible, due to the physical properties of the available isotopes which leads to the development of kinetically inert copper complexes (Zeng, Desai et al. 2013). Among the different copper radionuclides, ^{64}Cu with suitable decay characteristics is the most commonly utilized copper isotope for PET imaging. In addition, due to its 39% β^- emission, ^{64}Cu has also been used for targeted radiotherapy.

In this proposal, ^{64}Cu will be investigated as proof-of-concept study to validate the proposed purification strategy. Currently, ^{64}Cu is produced *via* the ^{64}Ni(p,n)^{64}Cu reaction on a biomedical cyclotron, and processing of the enriched ^{64}Ni target involves the dissolution of the nickel into 6 M HCl, cooling of the dissolved nickel solution, recovery of the nickel via ion chromatography, followed by elution of ^{64}Cu from the resin with 0.5 M HCl (McCarthy, Shefer et al. 1997). However ^{64}Cu obtained from such purification usually still contains a significant amount

of transition metal contaminants: Fe^{3+}, Ni^{2+}, Cu^{2+}, Co^{2+} and Zn^{2+}, as established by various analytic methods (Watanabe, Watanabe et al. 2009, Zeng, Lee et al. 2012, Zeng and Anderson 2013). Therefore by using a fluorous-tagged pyridinecarboxylic acid (PDA) extract, a highly efficient liquid-liquid extraction is proposed for the rapid purification of copper radionuclides. In addition, Dr. Zeng recently developed LC-MS quantification technology (Zeng and Anderson 2013) to quantify the overall effectiveness/purity of the process in this proposal.

1.1 MOLECULAR IMAGING: POSITRON EMISSION TOMOGRAPHY

Molecular imaging in oncology can be defined as "in *vivo* characterization and measurement of the key biomolecules and molecularly based events that are fundamental to the malignant state" (Kircher, Hricak et al. 2012). Molecular imaging plays a vital role in cancer management. Molecular imaging is a vital tool for cancer detection, staging, and assessment of effectiveness of the cancer treatment (tumor response, and etc.). Molecular imaging is a tool with the capacity to improve upon multiple aspects of cancer care. PET is a molecular imaging tool that characterizes and detects tumors based on molecular alterations (Kircher, Hricak et al. 2012). Images depicting the cancerous sites are obtained from PET through the decay emission detected by the instrument as shown in Figure 1. ^{18}F-fluorodeoxyglucose (FDG) is injected into the human body. FDG takes advantage of the Warburg effect. One of the characteristics of cancer cells is increased glucose metabolism and the conversion of glucose carbon to lactate. Increased glucose metabolism is due to the glucose transporters (gluT) increased expression and activity as well as

changes in the glycolytic enzyme expression and activity (Kircher, Hricak et al. 2012). Images are obtained via the detection of the positrons emitted by the radionuclide in the organ being studied.

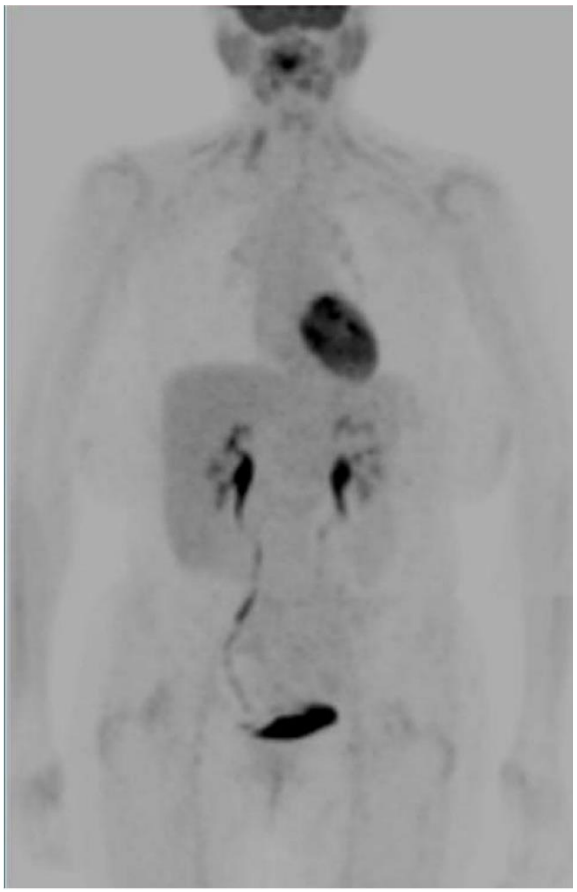

Figure 1. Normal PET Imaging (Miele, Spinelli et al. 2008)

There are direct imaging strategies and indirect imaging strategies. Positron Emission Tomography (PET) with the use of FDG to image falls under the direct imaging strategies. Table 1. depicts the properties of the radionuclide ^{18}F. FDG PET is based on the activity of the enzyme hexokinases (Blasberg and Tjuvajev 2003).The utilization of radionuclides with short half-lives has its disadvantages. There are advantages of the utilization of radionuclides such as ^{18}F- FDG

that have been widely studied and are currently approved of for the application in PET. One of the advantages is that the use of these radionuclides results in minimizing doses of exposure to the clinicians and patients. Also, multiple imaging studies can be carried out in the same day due to the short half-lives of the radionuclides, so the patient does not have to remain in the hospital overnight for the studies to be carried out (Holland, Williamson et al. 2010). However, due to the short half live (rapid decay) of the radionuclide, there are limits to the chemistry in regards to its use for radiopharmaceutical research. Radionuclides with emission of auger electrons and β- or α-particles are potential radionuclides for the design of radionuclide agents. Potential for future production of chemically identical agents for imaging and site directed radiotherapy exist for a variety of elements for which PET/therapeutic radionuclide pairs exist.

Table 1. Physical Decay Characteristics of the Cyclotron-Produced Conventional PET Radionuclides (Holland, Williamson et al. 2010).

Radionuclide	Half Life	Decay mode (% branching ratio)	Production Route	E(β+)/keV	B+ End-Point Energy/KeV	Abundance $I_{\beta+}$ / %	E_γ/KeV (intensity, I_γ/%)
^{18}F	109.77(5) m	β+ (100)	$^{18}O(p,n)^{18}F$ $^{20}Ne(d,\alpha)^{18}F$	249.8(3)	633.5(6)	96.73(4)	511.0(193.5)

1.1.1 Antibody based Positron Emission Tomography

Mesothelin (MSLN) is a protein-coding gene that is overexpressed in a wide range of cancers. MSLN is found in a majority of cancers such as: ovarian cancers, pancreatic adenocarcinomas, epithelial mesotheliomas, lung adenocarcinomas, gastric cancers, triple-negative breast cancers, uterine serous carcinoma, acute myeloid leukemia, cholangiocarcinoma, pancreatic adenocarcinomas, and in 100% of epithelial mesotheliomas. Due to the limited distribution of MSLN in normal tissues as well as its elevated expression in cancers, MSLN has great potential to be a suitable target for cancer diagnosis and therapy by using the specific antibodies for MSLN and the well-established coordination chemistry of copper. Copper-64 can be reacted using a chelator to the specific antibody for MSLN and thereby can be utilized in an antibody based Positron Emission Tomography system (Kobayashi, Sasaki et al. 2015).

1.2 COPPER 64

Copper offers a variety of radionuclides for imaging and radionuclide therapy. The various copper isotopes undergo β-, electron capture and positron decay, which make them of great interest. The variety of copper isotopes ranges from copper-60, copper-61, copper-62, copper-64, copper-66, copper-67. Copper radioisotopes have versatile characteristics like those of transition elements of 47 on the periodic table due to its range of oxidation states. In regards to the abundance of metals in the human body, after iron and zinc, copper is the third abundant metal in the human body. Due to its abundance in the human body, research and understanding of its pathways are known. This

information is vital for application of copper related systems in radiopharmaceuticals (Blower, Lewis et al. 1996).

1.2.1 Properties of Cu-64

The radioisotope of interest for this research is Cu-64. It has a half-life of 12.7 h, with an electron capture (41%) β^- (40%) and β^+ (19%) decays as well as auger electron emission with therapeutic potential. The half-life of Cu-64 tolerates for a sufficient time for radiopharmaceuticals synthesis of many complexes. Table 3. Shows the properties of many copper isotopes in which Cu-64 is one of the most versatile isotopes. Interest in Cu-64 increased due to the high yield and high specific activities from typical biomedical cyclotron (Blower, Lewis et al. 1996). ^{64}Cu has decent solubility in water with an oxidation state of +2 ($CuCl_2$) (Kim, Park et al. 2009). ^{64}Cu has strong chelating properties which is advantageous for labeling biomolecules through stable coordination complexes in formation with bifunctional chelators (Le, Howse et al. 2009). One of the many applications of Cu-64 is its potential use in PET as a radiotracer (Blower, Lewis et al. 1996). Based on the proposed application for which the radionuclide is intended for, it determines how the radionuclide or therapeutic agent is chosen. The diagnostic method is the deciding factor for choosing the radionuclide to work with (Holland, Williamson et al. 2010).

Table 2. Physical Properties of Copper Radionuclides for Imaging and Therapy. Ep – Energy of the most abundant penetrating (γ) radiation following the corresponding E_{np} Average Energy of the most abundant penetrating (β^+/β^-) radiation. R_{np} – Average range of the non-penetrating radiation in tissue (Blower, Lewis et al. 1996).

Radionuclide	$T_{1/2}$ (h)	Decay (%)	E_p (keV)	E_{np} (keV)	R_{np} (mm)	Source
^{60}Cu	0.38	B^+ (93) EC (7)	511 1332	873	4.4	Cyclotron
^{61}Cu	3.3	B^+ (62) EC (38)	511 283	527	2.6	Cyclotron
^{62}Cu	0.16	B^+ (98) EC (2)	511	1315	6.6	Generator/cyclotron
^{64}Cu	13	B^+ (19) EC (41) B^- (40)	511 1346	278 190	1.4 .95	Reactor/cyclotron
^{66}Cu	0.09	B^- (100)		1109	5.6	Reactor/cyclotron
^{67}Cu	62	B^- (100)	93	121	0.61	Reactor/cyclotron

Radioimmunotherapy (RIT) is a methodology that incorporates the use of antigen specific monoclonal antibodies (mAb) or mAb-derived reagents in an effort to deliver therapeutic radionuclides to tumor (Srivastava and Dadachova 2001). Studies have been carried out and ^{64}Cu-labeleled monoclonal antibodies are possible by the chelation of the mAb via a bifunctional chelator. Copper in itself binds weakly to proteins; therefore there is a need for a bifunctional chelator. The chelator will bond covalently to the antibody while also forming a strong bond with the copper (Anderson, Connett et al. 1992). Molecules like ^{18}F (half-life: 109.77 m) and ^{68}Ga (half-life: 67.71 m) would not be ideal for labeling due to their short half-lives in merely 2 – 3 hours. Their short half-lives would mean the activity of the radionuclides would have reduced significantly before optimal distribution of the radiotracer in the biological system. Elements such

as ^{52}Mn (half-life: 5.591 days) and ^{74}As (half-life: 17.77 days) won't be ideal for mAb labeling due to their extensive half-lives, which would take more than a day to decay, and would result in relatively poor counting statistics for PET and increase patient exposure to a higher radiation dose (Holland, Williamson et al. 2010) .

1.2.2 Current methods of production

^{64}Cu can be obtained from nuclear reactor such as ^{63}Cu(n,γ)^{64}Cu which relates to thermal neutron capture reaction. The radionuclide can also be obtained from a fast neutron reaction ^{64}Zn(n,p)^{64}Cu (McCarthy, Shefer et al. 1997). ^{64}Cu can be obtained from proton irradiation that is carried out in a medical cyclotron ^{64}Ni(p,n)^{64}Cu, ^{64}Ni(d,2n)^{64} (Kim, Park et al. 2009). The production of ^{64}Cu in regards to availability is readily available at the reactor sites but the specific activity of the resulting radioisotope is too low therefore, it cannot be used for labeling antigens or receptor targeted compounds. The high or low activity of the production of ^{64}Cu by reactor is dependent on whether the production is carried out by direct activation (n,γ) or indirectly through (n,p) on a Zn target (Blower, Lewis et al. 1996). Though the utilization of fast neutrons in the reactor results in ^{64}Cu with high specific activity, the ^{64}Cu is not readily available, which makes using it not as feasible and time and cost intensive (Blower, Lewis et al. 1996).

The preferred method of ^{64}Cu preparation is via biomedical cyclotron (McCarthy, Shefer et al. 1997). The irradiation of enhanced ^{64}Ni target carried out in a cyclotron results in the highest specific activity in comparison to the reactor produced ^{64}Cu (Blower, Lewis et al. 1996). What is currently in use for the production of ^{64}Cu, is ^{64}Ni target and ^{68}Zn target. The ^{68}Zn(p,α,n) allows for the production of both ^{64}Cu and ^{67}Ga and the target is cheaper in comparison to the ^{64}Ni target.

But this method results in a product that requires radiochemical separation that is complex with a high contamination probability from a variety of radionuclide impurities. Also, the ^{64}Cu yield from the ^{68}Zn target is preferential. The alternative cyclotron method advantage is that the reaction requires a low proton energy (<15MeV) in comparison to the alternative, which requires (30 MeV). The ^{64}Cu from the ^{64}Ni target is higher in yield and has a lower potential for impurities due to the fact that the reaction pathway results in fewer side reactions. Its disadvantages are that the process requires enriched ^{64}Ni target, which is expensive, and it's not as applicable when using a larger cyclotron. The larger weight of the ^{64}Ni makes the process ineffective due to the cost and the need to design a target specific system (Le, Howse et al. 2009).

1.2.3 Current methods of purification

As previously stated, in order to effectively utilize the radionuclide, contamination has to be minimized. The contaminants compete with the Cu-64 while producing the radiotracer, especially nickel. If applied to a human subject, the nickel increases the stress on the human body and may cause various types of acute and chronic disorders. These disorders affect the liver, lung and kidney and include the following: gastrointestinal distress, pulmonary fibrosis, and renal edema. Cu-64 separation from a nickel target is usually separated by the means of solvent extraction, precipitation, electro plating and ion exchange. Ion exchange is widely used because unlike the other methods, it eliminates the need for added carriers (Fan, Parker et al. 2006). Most ion exchange methods utilize a base anion exchanger. Cation exchangers are not utilized due to the selectivity coefficient of the cation exchangers being very close to that of metal ions of the same charge. While ion exchange in general may lead to the use of fewer chemicals in comparison to

solvent extraction, the process also has its limitations. Anion exchange uses a high degree of chemicals and after separation; there is a high content of residual nickel in the radioactive product stream, which is harmful to human health. There is a need for a method that reduces the amount of residual nickel in the product stream, specifically a method that purifies Cu-64 with a purity of > 99 % (Fan, Parker et al. 2006).

1.2.4 Methods of quantifying metal impurities

Radiometals are known to have a significant amount of metal impurities that at specific levels impedes the radiolabeling. Impurities must be minimized to achieve high specific activity. High specific activity is important for imaging agents that target proteins. Dr. Zeng Research Group has identified the Liquid Chromatography Mass Spectroscopy as an effective means to quantify non-radioactive metal contaminants and also as a means to determine Effective Specific Activity for the chelator of interest, thereby resulting in an optimized relabeling condition (Zeng and Anderson 2013).

1.2.5 Selective extraction

Solvent extraction, in conjunction with chelators, is used in an array of fields. Commercial chelating entrants are readily available and are used in combination with organic diluents. A concern in using liquid-liquid extraction is the environmental impact of the use of a high volume of volatile organic diluents. The use of a membrane aids in the minimization of the use of organic solvents. Alkylated pyridinecarboxylic acid derivatives play a major role in separation chemistry;

one major reason for this is that the carboxylic acid group is present on the chelator. (Tasaki, Oshima et al. 2007)

1.2.6 Chelator

Successful purification of copper 64 is dependent on extraction of copper from the bulk aqueous solution of metal ions. A selective chelator is key to successfully extracting copper (a preferential chelator is one with an affinity for copper and the organic phase over the aqueous phase). A potential candidate is EHPA (A (N-6-(2-ethylhexylamido) - 2pyridinecarboxylic acid). Separation of copper (II) can be carryout using EHPA at a low pH (acidic environment). As seen in Figure 2, 95.5% extraction of copper (II) was carried out at a pH of about 2.90, based on the preliminary data by Tasaki et al. Based on the experimental results, it is likely that the alkyl amide group does not participate in the formation of a complex with copper (II). The formation of the EHPA copper (II) complexes is hypothesized to be via the pyridine moiety and carboxylic acid (to form a five membered ring). The affinity for EHPA for divalent metals ($Cu >> Zn \approx Pb \approx Ni \approx Co \approx Cd \approx Mn$) can be explained using Pearson's HSAB (Hard, Soft, Acid, Base) concept. EHPA's greater affinity for copper (II) is due to copper's ability to form a tetra-coordinate geometry which is a neutral complex in comparison to the octahedral coordinate complex that other metals such as Zn, Co and Mn form with EHPA (Tasaki, Oshima et al. 2007).

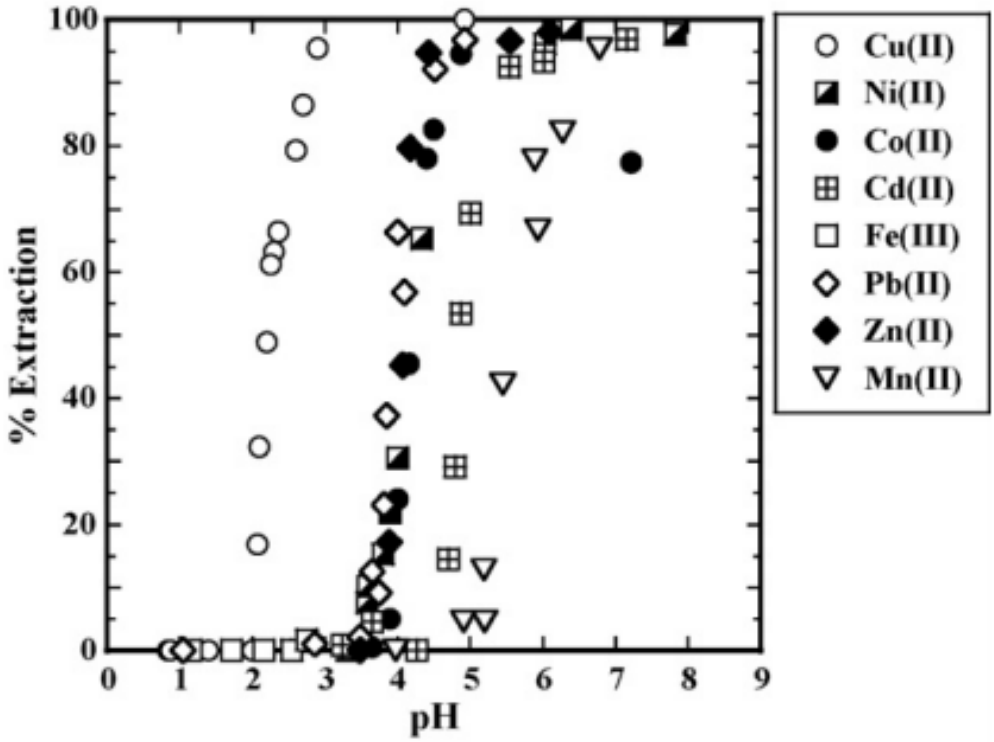

Figure 2 Effect of pH on the percentage of metal ions with EHPA.

2.0 MATERIALS AND METHODS

For the purpose of this research, lab experiments were carried out in Dr. Gao's lab located on the 9th floor of Benedum within the chemical engineering department at the University of Pittsburgh. Occasionally, some experiments, such as the Ultraviolet visible (UV-Vis) spectroscopy studies, and test for solvent soluble dye, were carried out in Dr. Zeng's lab located within the Center for Biotechnology and Bioengineering, fourth floor of the bridge side building located within the University of Pittsburgh.

2.1 WATER-OIL SEPERATION DEVICE

2.1.1 Silicon Membrane Holder

The structure used to house the membrane was designed using SYLGARD® 184 SILICONE ELASTOMER KIT.

1. 10:1 Parts of the polymer were mixed and then placed in a desiccator for approximately 2 hours until there were no more bubbles.
2. The mixed polymer was placed into a mold and heated at 80℃ on a hot plate for approximately an hour, then allowed to cool down.

3. The mixed polymer was placed into a mold and heated at 80℃ on a hot plate for approximately an hour, then allowed to cool down.
4. The pieces needed to create the designs were cut from the polymer block (Figure 3).
5. The pieces were cleaned using ethanol and dried using Nitrogen gas.
6. The surface of the cut pieces was further cleaned using Plasma Cleaner / Sterilizer PDC 32 G (Figure 14.) for 30 seconds and then immediately the pieces were joined together and placed in the oven for 20 – 30 minutes to ensure adhesion. Figure 2B depicts the final holding membrane made from the silicone.

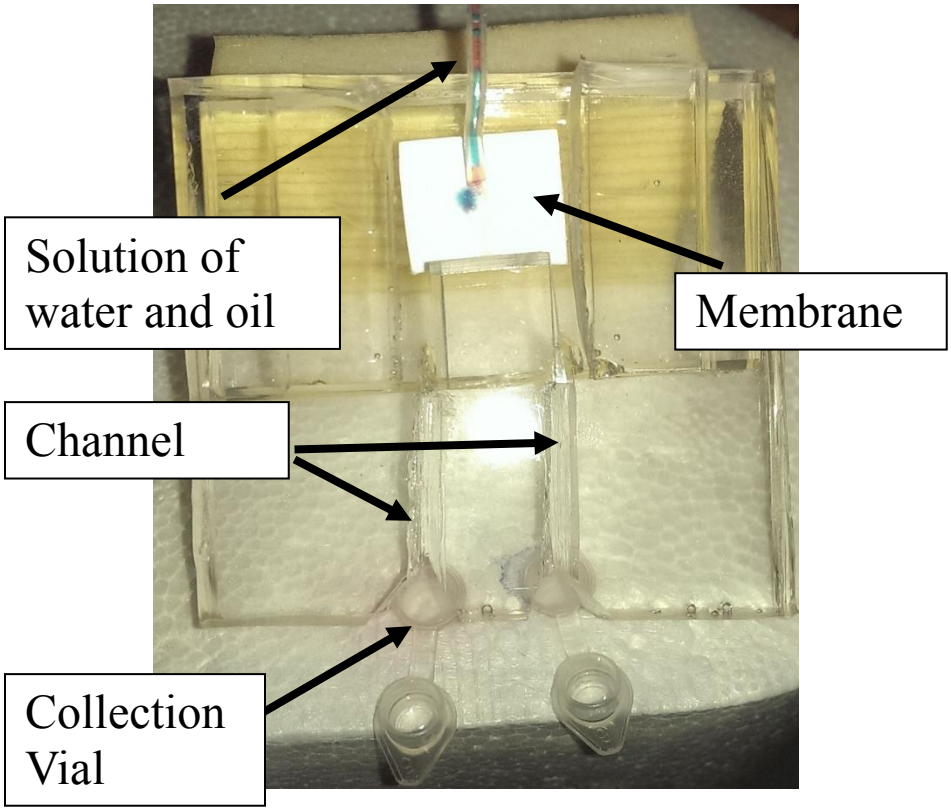

Figure 3. Final setup of the structure in which future modifications were modeled after and innovated upon.

2.1.2 3-D printed Membrane Holder

SolidWorks Model was implemented in the design of the membrane-holding device. Figure 4A depicts the drawing of the holding device utilized as a holding chamber for the membrane utilized in the experiments discussed further in this paper. The device was 3-D printed at the Swanson Center for Product Innovation located at the University of Pittsburgh. Figure 4B illustrates the 3-D printed device and its functionality.

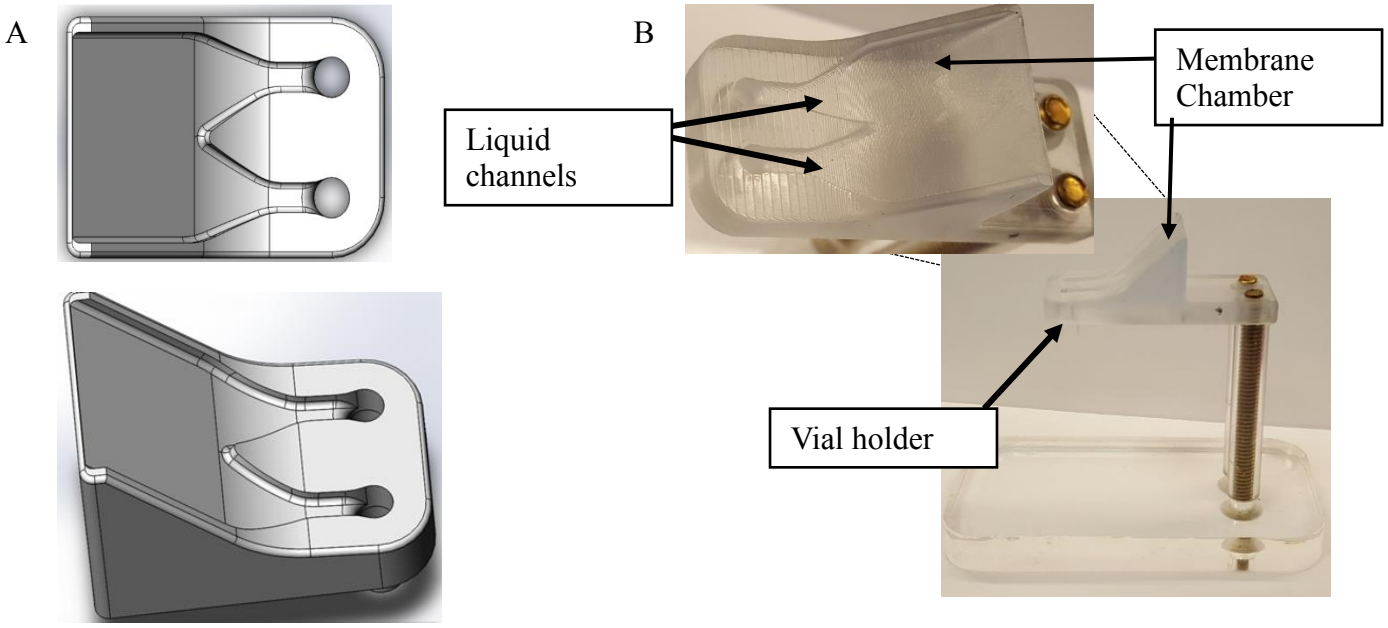

Figure 4. (A) SolidWorks model of the water oil separation device (B) 3-D printed image of the water-oil separating device.

2.2 FUNCTIONALIZING THE MEMBRANE

The membrane utilized in the separating device, was functionalized using two commercially available products named No. 1 and No. 3. Half of WhatmanTM Filter Paper 3 (CAT No. 1003-055) was functionalized by hand dipping the filter paper in the hydrophobic solution (No.1) and then immediately being placed in an oven at 85℃ overnight. Then the other half was hand dipped in the hydrophilic solution (No.3) and placed in an oven set at 85℃ overnight. Figure 5 is the image of the functionalized membrane.

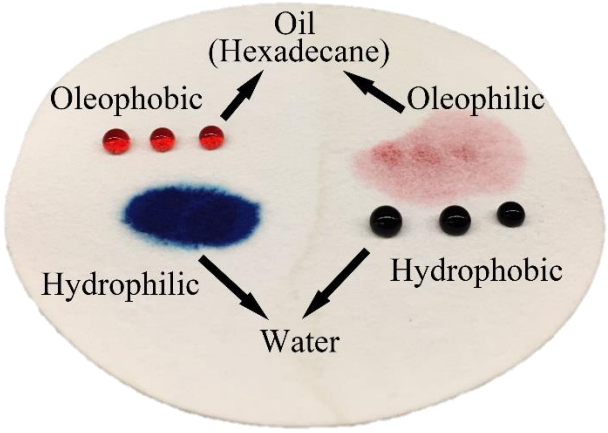

Figure 5. Functionalize membrane with an oleophobic area and a hydrophobic area. Red fluid depicts the organic phase and the blue fluid depicts the aqueous phase.

2.2.1 Flow Rate Studies

After functionalizing the membrane used for this study, then the solutions were prepared. The aqueous solution was made by dissolving 10 mg of water-soluble dye, methylene Blue (CAS 7220-79-3) in 10 ml of nano-pure water. The organic solution was made by dissolving 12.5 mg oil-soluble dye, red O (CAS 1320-06-5) in 12 ml of toluene. Figure 6 depicts the lab setup for how the various studies were carried out.

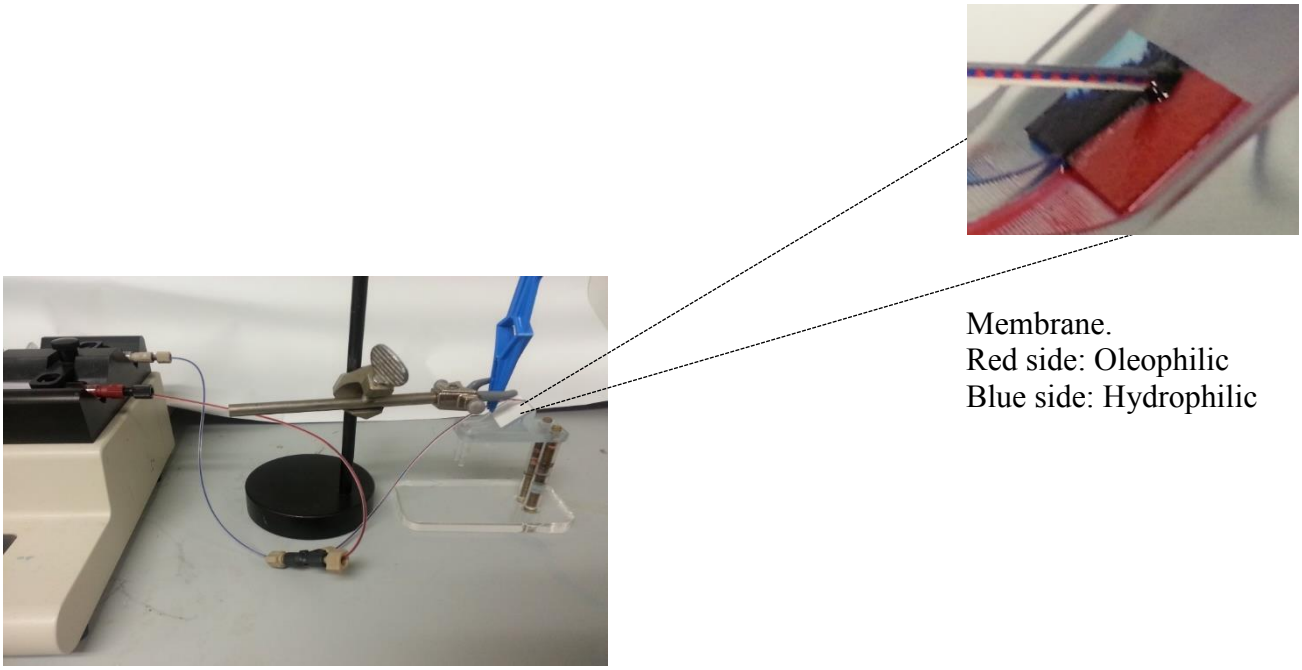

Figure 6. Schematic of the flow rate studies experiments.

2.2.2 Capacity Studies

Membrane capacity studies were carried out by having a fully oleophobic functionalized membrane and a fully hydrophobic functionalized membrane of various sample areas: 22.9, 8, 6, 4, 2, 1, .25 cm^2. Each membrane was fully saturated and the weight difference was tracked for each type of membrane**.**

2.2.3 System Optimization Studies

A series of dyes made in Dr. Zeng's lab were tested. The purpose was to determine the proper dye that is partially soluble in the aqueous phase (1:9, Dimethyl sulfoxide (DMSO) : nano-pure water) but has an affinity for the organic phase (toluene). The dye being tested was dissolved initially in DMSO in a vial and then diluted with water. Then an organic phase was added to the vial and agitated using a sonicator. The emulsified solution was separated by the use of a centrifuge. The determining factor to verify if the dye was extracted in the organic phase was observed via a color change in the organic phase.

Procedures followed:

- First emulsifying a solution of toluene and the water-dye mixture in a glass vial.
- Let the solution sit unperturbed for a few minutes.
- Extract the parts of the solution that are not emulsified (using a pipette).
- Run the emulsified part of the solution through the functionalized paper towel.

- Test the hydrophobic paper towel and the oleophobic paper towel. (This will help isolate whether or not the functionalized paper is breaking the emulsion or absorbing the solution. This will also help determine if what the emulsion consists of in regards to whether it's purely toluene or water.

3.0 RESULTS AND DISCUSSION

Findings from these experiments as stated in the preface were mostly qualitative data that requires further intensive study to properly quantify and qualify the effectiveness of the system through the use of liquid Chromatography, mass spectroscopy, ultraviolet visible spectroscopy studies. This work did confirm the efficiency of the membrane in separating the aqueous solution from the organic solvent. Based on the data obtained from UV-Vis spectroscopy of the separated two phases that contain different colored dyes, the purity of either the water or the organic phase separated by the membrane is greater than 99 %.

3.1 PRELIMINARY STUDIES RESULT

Human error significantly affects the effectiveness of the design in respect to separating the water and oil. If the polymer does not adhere to each other properly and there is a slight leak anywhere, the oil substance will make its way all over the structure, which indirectly affects the results. The membrane has to be saturated before separation will take place at a lower flow rate (50 ≤flow rate <100 ul/min). As the flow rate increases (100 ≤flow rate ≤200 ul/min) the size of the platform seems to play a major role in separation.

At a higher flow rate the solution races down the membrane faster and at impact on the platform, it either falls to the left or the right side into the appropriate channel (minimizing the platform will reduce the effect of liquid going into the wrong channel). In regards to designing the 3-D model, some of the takeaways from the preliminary studies were: the structure has to be at an acute angle, 45 ° – 85° would work. But the difference in the degree is hard to observe due to the lack of repeatable results.

3.2 CAPACITY AND FLOWRATE STUDIES

Results from the studies showed that the functionalized hydrophilic membrane in general had a greater carrying capacity than that of the oleophilic membrane. Therefore, during the flow rate studies we expected to have the organic phase drop first before the aqueous phase. But this was not what was observed. This can be attributed to the fact that the hydrophilic membrane had a greater wettability than the oleophilic membrane. This resulted in a faster absorbance of the aqueous phase into the membrane, as seen in Figure 7**.** These findings resulted in a shift from the commercial solvent number 3 to a different product (FBL035) as a means to functionalize the membrane and make it oleophilic. The new product proved to be more effective and resulted in a similar wettability in the oleophilic membrane as the hydrophilic membrane.

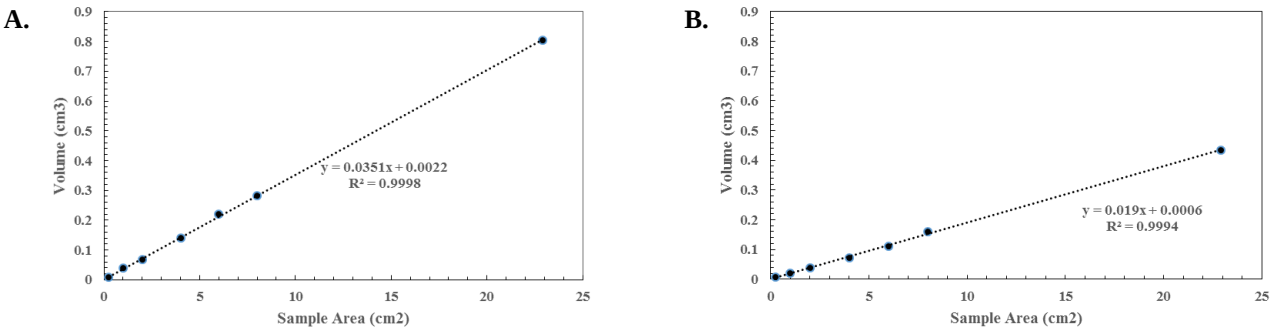

Figure 7. (a) Shows the carrying capacity of the membrane hydrophilic membrane. (b) Shows the carrying capacity of the oleophilic membrane.

Flow rate has an effect on separation speed. As the flow rate increases, the time needed to carry out the separation decreased as presented in Figure 8. Based on the data obtained from ultraviolet–visible spectroscopy (UV-Vis) of the separated two phases that contain different colored dyes, it was determined that the purity of either the water or the organic phase separated by the membrane is greater than 99 %. This led to the conclusion that flow rate only had an effect on the speed of the process but not the effectiveness.

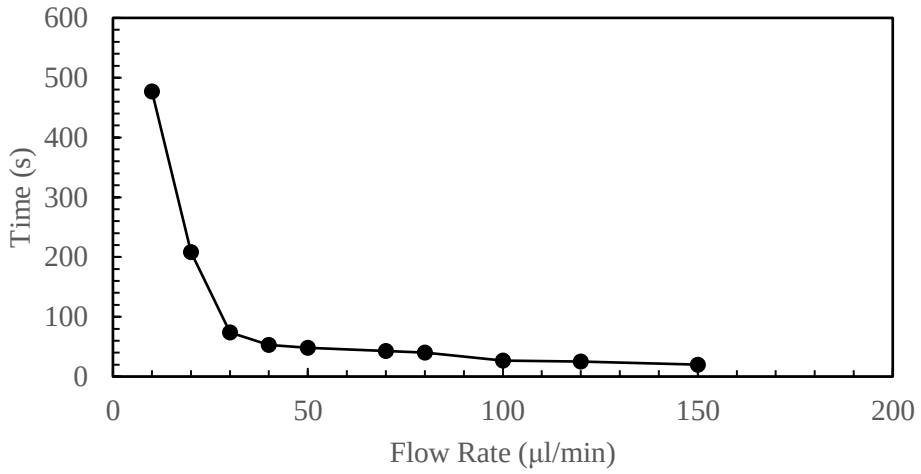

Figure 8. Shows the effect of flow rate on the separation process

3.3 SYSTEM OPTIMIZATION STUDIES

In this study we found that there is no need to use a dual functionalized membrane system. Commercial paper towels were functionalized and a new system was employed.

3.3.1 Dye Extraction Studies

Studies carried out to test the fluorine tagged dye with the hydrophilic functionalized paper towels showed that the emulsified phase is indeed an aqueous phase and the emulsified liquid is not being broken up by the fictionalized membrane, as seen in Figure 9**.** Running the emulsified solution mixed with toluene through the membrane does not result in extraction of the dye from the

emulsified solution into the organic phase. The proper way to ensure extraction is to first centrifuge the solution before running it through the functionalized membrane. The fluorine tagged dye, even after being centrifuged, was not extracted into the organic phase. There was a faint color change, which is indicative of slight solubility of the fluorine tagged dye in the organic phase.

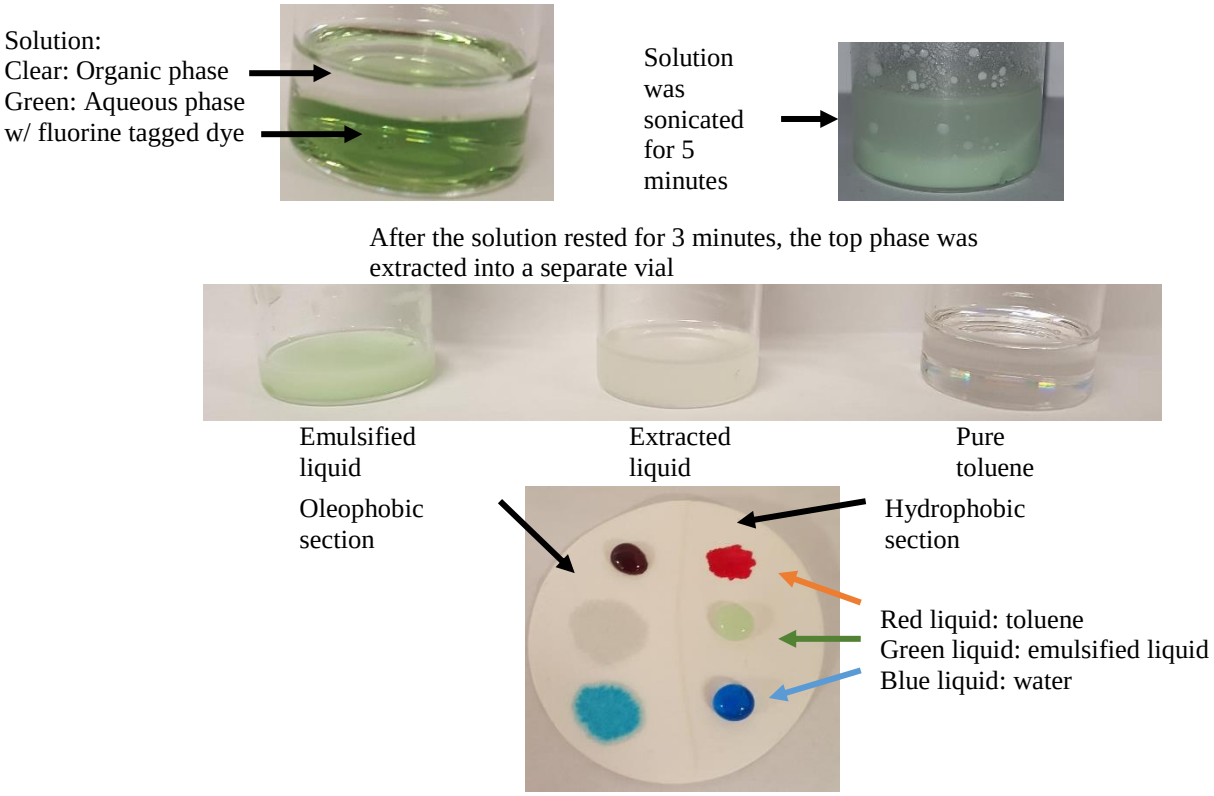

Figure 9. Figure depict the extraction study of the fluorine tagged dye

If a centrifuge step is not carried out, in order to break the emulsion and extract the dye into the organic phase, a different method needs to be employed to separate the emulsion. The red O dye was tested and was partially soluble in DMSO and after being sonicated and then centrifuged, the red O was successfully extracted into the organic phase as seen in Figure 10.

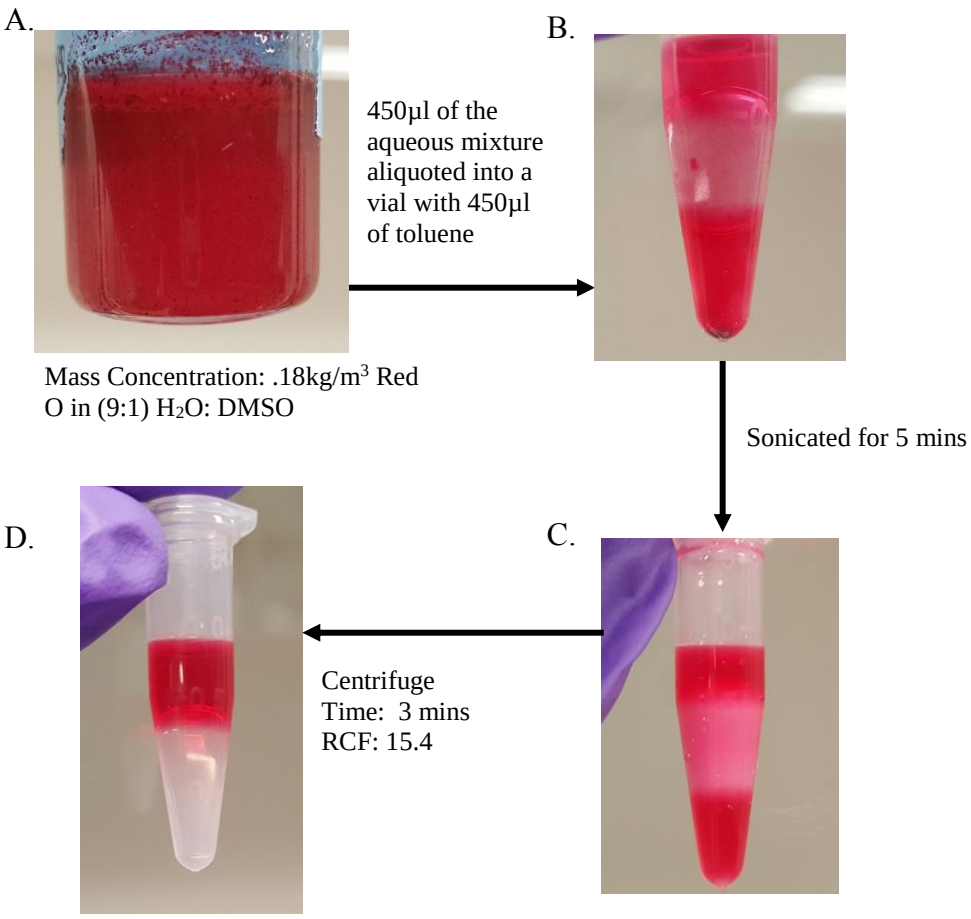

Figure 10. Depicts the Red O dye extraction studies (A) Shows the solution with particles of undissolved Red O dye (B) Two phase system depicting the initial phase of Red O dissolving in toluene (C) Emulsion forms in the organic phase after sonication (D) Red O completely in the organic phase (toluene) after the solution has been centrifuged

4.0 CONCLUSION

Thus far we have a proven system that is able to separate oil and water with a high efficiency. The next phase of this research will be in the utilization of this separation technique to separate Copper (II) from a solution of Fe^{3+}, Ni^{2+}, Cu^{2+}, Co^{2+} and Zn^{2+} by combining the use of fluorine-tagged PDA derivatives with the membrane-based separation process. The proposed fluorine-tagged PDA derivatives will be synthesized by coupling 2-pyridinecarboxylic acid with commercially available fluorous alcohol, and the resulting fluorine-tagged PDA derivatives will be tested at various pH and solvent modification ratios to find the optimal chelator as well as the optimal conditions (concentrations and solvent ratio s) for copper extraction. Once success has been made in extracting non-radioactive copper from the transition metals that exist in the cyclotron-produced ^{64}Cu, we will test the efficiency of our technique by using copper radionuclides. The metal contaminants in ^{64}Cu before and after the proposed purification process will be quantified by using the LC-MS approach developed by Dr. Zeng's research group (Zeng and Anderson 2013). Automation of this liquid-liquid process will lead to lower radiation doses for radio chemists. It will reduce cross-contamination risks and overall it will lead to a more efficient purification method compared to a solid phase extraction process.

5.0 FUTURE WORK

A comprehensive understanding of copper isotopes has not yet been fully met. Thus far the chemistry of copper that has been studied is limited to the chelators and functionality/ complexes of the ligands studied (Blower, Lewis et al. 1996). Better understanding of the copper isotopes, especially copper 64, is needed so that research, such as the research carried out in this paper by Tasaki et al, can be specifically designed in order to ensure that the system is optimized for enhanced copper 64 performance. ^{18}F-FDG PET imaging has been the utilized for over 20 years, therefore the introduction of a new radiotracer (Blasberg and Tjuvajev 2003) will need to be a well-defined and validated model that will perform well above the current radiotracer by being site specific with enhanced images.

5.1 CHELATION STUDY

Based on preliminary research carried out by Tasaki et al, PDA derivative, EHPA (N-6-(2-ethylhexylamido)- 2pyridinecarboxylic acid) will be used in the extraction of copper into the organic phase (Tasaki, Oshima et al. 2007) while other transition metals will remain in the aqueous phase under strong acidic conditions (pH=2). The extracted copper can be further released into the aqueous under strong acidic conditions (pH<2). As seen in Figure 10A, with a

coordination number of 6, the pyridine moiety and carboxylic acid play a vital role in the EHPA chelation of copper. The objective of my research is to develop an automated liquid-liquid extraction process for high-efficiency rapid purification of copper radionuclides by combining the use of a fluorine-tagged PDA extractant with a membrane-based water-oil separation process. The process comprises 3 steps as shown by Figure 10B. : (i) chelate copper radionuclides with fluorine-tagged PDA in aqueous solution at moderate acidic conditions (pH>2), (ii) extract chelated copper radionuclides from aqueous phase to organic phase, and (iii) release copper radionuclides from fluorine-tagged PDA in organic phase back into aqueous phase under strong acidic condition (pH <2). In both the 2nd and the 3rd steps, the organic phase and the aqueous phase will be separated by using a membrane to facilitate the automation process.

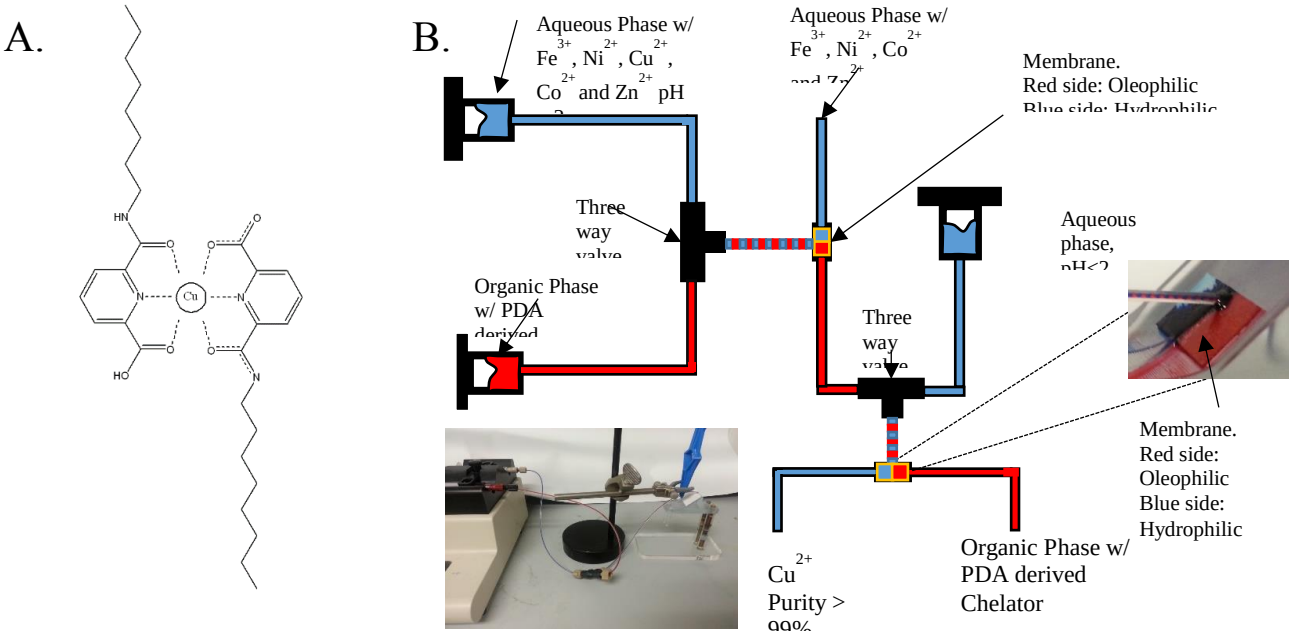

Figure 11. a) Complex of EHPA with Copper and b) Schematic of the purification process of copper.

APPENDIX

PRELIMINARY RESULTS

Appendix includes images from the preliminary studies carried out to optimize the membrane-carrying device for the water-oil separation process.

Figure 12. Shows the membrane inserted into the holding structure

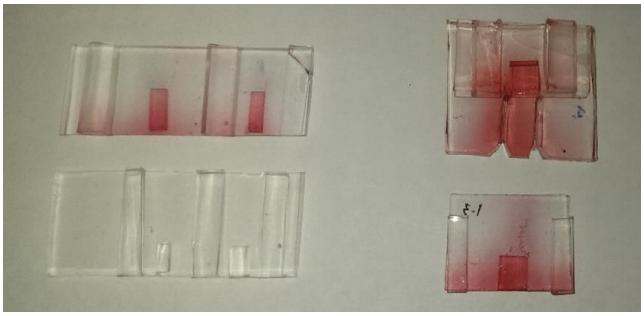

Figure 13. Variations of structures that were designed as a means to test the effect of the platform width and channel size to determine which parameter is optimal.

Figure 14. The Plasma Cleaner used in cleaning the surface of the polymer used to create the holding structure in this experiment.

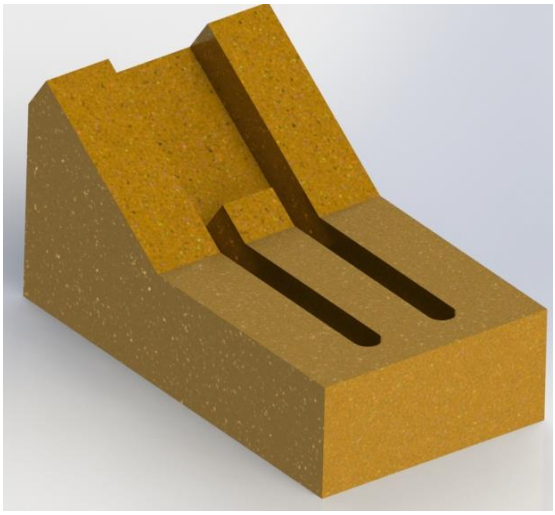

Figure 15. The SolidWorks Model of the ideal holding structure that will be implemented in the next stage of the design.

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